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Review

The genetics of epilepsy—The past, the present and future

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ABSTRACT

A brief history of human genetics: Sixty years is an appropriate yardstick for many reasons, not least for the remarkable advances in medicine, public health, psychology and biological disciplines. Particularly relevant is the approaching 60th anniversary of the discovery of the structure of DNA, which unlocked the driving force of nature and spawned a plethora of scientific discoveries and economic development through the Bitoech industry. Prior to 1953, and before Watson and Crick burst into the Cambridge pub with their eureka moment, it was known that chromosomes were important, the first principles of clinical cytogenetics were emerging and the rules of heritable traits were well-advanced, but without the basic framework or mechanism. Human Molecular Genetics arrived when the first mutations were linked to human disorders reflecting the advances in understanding the genetic code, assembly of protein building blocks and methodological advances in reading the physical code (all be it very difficult process at the time). Accelerated by the introduction of recombinant gene technology in the 1980s, and in conjunction with the development of linked genetic marker maps, the catalogue of genes associated with disease has risen exponentially with classical examples such as sickle cell disease, cystic fibrosis and Huntington's disease. The advances approached super-sonic dimensions when genes were found in Mendelian families, and mapping strategies were adopted using the variation map of the human genome (SNP's, di-nucleotide repeats), in addition to targeted candidate gene approaches aided by the significant database resources available to investigators. Super-sonic gave way to light-speed with the publication of the 3 billion letters of the genetic code which constitutes the human genome, followed quickly by genomes in plants, bacteria, pathogens, fruits and vegetables, and a menagerie of eukaryotic and prokaryotic animals, often representing model systems for genomic and pathophysiological research. In short don't blink or you'll miss the next revolution – too late, it's just happened!

1. Epilepsy genetics—where are we?

The past 15 years has witnessed a golden age of gene-discovery in idiopathic and syndromic epilepsy. Unlike other complex neurological or psychiatric conditions, idiopathic epilepsy enjoys the privilege of informative Mendelian autosomal dominant (AD) families that provide a direct insight into the neurobiology of the condition. The compendium has grown to around 21 genes in idiopathic generalised epilepsy (IGE), the vast majority of which are channelopathies or regulate the action of excitatory or inhibitory neurotransmission in the central nervous system (CNS) (Table 1). Other genetic insights have been gained through the discoveries in syndromic epilepsy and in conditions where epilepsy is co-morbid with other disorders such as broad-spectrum encapthalopathies, learning difficulties, psychiatric conditions and cortical migration disorders. Despite this success, one of the limitations of these discoveries is the lack of impact from a wider population-wide perspective, with the notable exception of *SCN1A* and Dravet syndrome and *LgI1* in well-defined temporal lobe epilepsy with auditory features. The ascertainment of the dominant or X-linked families has created a bias of discovery in favour of receptor subunits, because they exert their effects courtesy of compromised multi-meric ion channels that are particularly susceptible to dominant modulation of channel function. Despite this extraordinary period of gene-discovery and neurobiological mechanisms of epileptogenesis, it remains that the majority of epilepsy patients have no genetic explanation and this represents one of the many challenges for future epilepsy research.

2. Dr. Mendel and familial epilepsy

The principles of Mendelian inheritance have been the driving force for epilepsy gene-discovery. Since 1995 there has been a steady stream of genes identified in familial idiopathic epilepsy[1–4] and was made possible by pedigrees that typically follows a

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Table 1
Genes associated with familial idiopathic epilepsy.

Gene symbol	Epilepsy phenotype	OMIM	Chromosome
Na channel			
SCN1A	GEFS+, SMEI	607208	2q24
SCN1B	GEFS+	604233	19q13.1
SCN2A	BFNIS	604233	2q23
Ca channel			
CACNA1A	IGE, EA, SCA6, FHM	600669, 108500	19p13
CACNB4	IGE, JME	600669, 606904	2q22–23
CACNA1H	CAE	607682	16p13.3
Ach receptor			
CHRNA4	ADNFLE1	600513	20q13.2–q13.3
CHRNB2	ADNFLE3	605375	1p21
$GABA_A$ receptors			
GABRA1	JME	606904	5q34
GABRG2	GEFS+, CAE	604233, 607681	5q34
GABRD	GEFS+	604233	1p36.3
Cl channel			
CLCN2	CAE, EGMA	607682, 607628	3q26
K channel			
KCNQ2	BFNC1	105370	20q13.3
KCNQ3	BFNC2	121201	8q24
LGI1	ADPEAF	600512	10q24

The various channelopathies associated with idiopathic epilepsy affecting both inhibitory and excitatory receptor systems. OMIM numbers for each gene are detailed for more in-depth reference.
Genes: *SCN1A*: sodium channel α1 subunit; *SCN1B*: sodium channel β1 subunit; *SCN2A*: sodium channel α2 subunit; *CACNA1A*: Voltage-dependant P/Q Type Calcium channel α1; *CACNB4*: Voltage-dependant Calcium channel β4; *CACNA1H*: Voltage-dependant T-type calcium channel α1H; *CHRNA4*: acetylcholine receptor subunit, α4; *CHRNB2*: acetylcholine β2 receptor subunit; *GABRA1*: $GABBA_A$ receptor α1 subunit; *GABRG2*: $GABBA_A$ receptor γ2 subunit; *GABRD*: $GABBA_A$ receptor δ subunit *CLCN2:* chloride channel gene 2; *KCNQ2/KCNQ3*: potassium channel subunits; *LG11*: leucine-rich, glioma inactivated 1 gene.
Syndromes: ADNFLE: autosomal dominant nocturnal frontal lobe epilepsy; BFNC: benign familial neonatal convulsions; $GEFS^+$: generalised epilepsy with febrile seizures plus; SMEI: severe myoclonic epilepsy of infancy; CAE: childhood absence epilepsy; FS: febrile seizures; BFNIS: benign familial neonatal-infantile seizures; ADJME: autosomal dominant juvenile myoclonic epilepsy; IGE: idiopathic generalised epilepsy; ADPEAF: autosomal dominant partial epilepsy with auditory features; JME: juvenile myoclonic epilepsy; EGMA: epilepsy with grand mal upon wakening.

Mendelian inheritance pattern and where the affection status of the pedigree members is well-defined. These patterns in large families are often AD with reduced penetrance but with some recent examples of recessive and X-linked inheritance in cryptogenic epilepsy yielding new genes in consanguineous families.[5,6] That said, the vast majority of familial epilepsy has an undetermined inheritance pattern and often does not reach the statistically demanding limits of parametric linkage experiments. Therefore, informative families from genetics perceptive are certainly rare, but remain powerful and relevant for indentifying genes that are informative for the private family and become immediate candidate genes for further analysis in families and unrelated populations with the same phenotype. In addition, any new gene spawns frantic activity amongst scientists who chase the neurobiological mechanism underling the epilepsy – ranging from specialised electrophysiology to animal model experts. More importantly, great care must be taken with families who are consented into research since the investigation of the 'epilepsy family' can have both profound positive and negative outcomes as revealed by Hammond et al. in this edition of Seizure.

3. An avalanche of channelopathies

The majority of genes detailed in Table 1 represent subunit components of multi-meric receptor channels which mediate K^+, Na^+, Ca^{2+}, and GABA voltage-gated/ligand-gated neurotransmission or ensure neuronal homeostasis/function. The majority of these ion channel genes are generally segregated into mediating excitatory or inhibitory neurotransmission and normally found in rare families where dominant mutations segregate with the epilepsy phenotype, although there can be heterogeneous intra-family expression of the phenotype. In most cases, each gene mutation has functional validation of pathogenicity and the lack of a functional evidence-base should be viewed with suspicion and the genotype not regarded as a mutation, but rather as a rare variant, even if there is significant segregation of a gene variant with the phenotype in any given family.

Confirmation of a pathogenic mutation usually involves either a proof-of principle in vitro assay or patch-clamp electrophysiology, often in association with convergent data from sophisticated clinical testing, advance medical imaging and animal models of epilepsy that may involve analogous genes. As a result the main pathogenic mechanism to emerge which leads to over-excitation and synchronous firing of neural networks is mirrored in other channelopathy disorders. These can be (i) dominant missense mutations that will compromise the channel current dynamics or affect the efficacy of ligand-binding, (ii) rare dominant missense mutations that lock the receptor into a permanently open state of tonic activity, or (iii) dominant and recessive nonsense mutations that can trigger haploisufficency or nonsense-mediated degradation of stop codon and frameshift polypeptides, leading to a reduction of cell surface expression of the receptor.[7] All conspire to either increase excitability of neural networks or withdraw the inhibitory tone in neurons and inter-neurones, with seizure events determined by which networks are affected in conjunction with clinically relevant issues around seizure initiation sites and spread of synchronous firing.[8,9]

(a) Excitatory ion channel genes: These include the sodium, nicotinic acetylcholine and calcium channel genes which are involved in BFNIS, ADNFLE, GEFS+, SMEI, JME and CAE epilepsy sub-types. These channels belong to large gene-clusters which contribute to the pathogenesis of other neuronal/electrical conduction disorders such as long QT syndrome, Brugada syndrome, hemiplegic migraine and paroxysmal paralysis cases.[10] In particular, mutations in *SCN1A* in SMEI represents the best example, to date, of a genetic finding in familial epilepsy that has translated into a population-based diagnostic test. From discovery of 600 mutations in SCN1A, approximately 90% arise as *de novo* events and around half of these results in protein truncation and haploinsufficiency (one intact gene-copy is not sufficient to rescue the phenotype). Latest research confirms that the timing of these *de novo* mutations can occur at any time between parental gamete formation (germ-line) and late premorula stage of the embryo (event in affected case).[11] The discovery of *LGI-1* mutations in autosomal dominant lateral–temporal lobe epilepsy with auditory features (ADPEAF) presents another example of the transfer from families (and animal models) to sporadic population based cases with same phenotype. This was hailed as the first non-channelopathy mutation in IGE, which in terms of gene family is correct. However, functional characterisation of this cancer susceptibility gene revealed that it was a coupled protein partner of K^+ Channels directly related to channel function[12] and recently shown to impair postnatal development of glutamatergic circuits.[13]

(b) Inhibitory ion channels genes: These include the potassium (K^+), and chloride channel (Cl^-) genes which are associated with BFNC1 and 2, EA1, JME, CAE, GEFS+, and ADPEAF epilepsy phenotypes. The principal role of K channels is stabilization of the cell membrane potential by terminating, dampening and lowering excitatory inputs into the cell. It is logical that removal of this influence may trigger indiscreet synchronous neuronal-network firing in epilepsy, although mutations in *KCNQ2* and *KCNQ3* result

in a benign neonatal form of seizure predisposition and is developmentally restricted to within the first months of infancy. The principal role of Cl^- channels is to mediate fast-response inhibition and include the $GABA_A$ and Glycine receptor systems. Mutations in $GABA_A$ subunits ($\gamma 2$, $\alpha 1$, and δ) are associated with GEFS+, CAE and JME, although only in small proportion and with disappointing effect on a population basis. Glycine receptor mutations cause human hyperekplexia, a non-epileptic paroxysmal disorder, which can often cause anoxic seizures and brain damage in neonates.[14]

4. Epilepsia syndromica

Syndromic epilepsy is a wider source of genetic discoveries in disorders where epilepsy is co-morbid with other pathology but is not necessarily the most morbid factor of the core presentation. Examples include syndromes with learning difficulties (LD) and cognitive decline, migraine, cortical malformation disorders and mitochondrial/metabolic disorders. Some recent examples are given below, but this is by no means a full compendium of genes and disorders in this category.

(a) Learning difficulties and intellectual disabilities – autosomal and X-linked: Epilepsy is one of the most common secondary disabilities in people with cognitive impairment and learning difficulties, the prevalence increasing with the severity of the intellectual disability. About 50% of those with profound learning disability and between 10% and 20% of those with mild disability suffer from lifetime seizures. Epilepsy is thus an important indicator of underlying cerebral dysfunction and the aetiologies for syndromic and non-syndromic cognitive impairment are varied and involve a wide spectrum of autosomal and X-linked genetic determinants (over 300 genes – see [15,16]). This strongly suggests that cognitive impairment and LD can emerge within a common pathway of many different types of abnormal cellular processing with no one overriding or dominant mechanism. Some of these mechanisms include defects in neuronal signalling molecules, components of vesicular and neuronal architecture/dynamics, transcription factors and enzymatic regulation. Undoubtedly, there are more genes to be identified and all have the potential to unveil an epileptogenic mechanism and become a target for further research in IGE.

(b) Neuronal migration disorders: The majority of patients showing neuronal migration disorders in cortical structures suffer from pharmaco-resistant epilepsy. The introduction of non-invasive MRI imaging of the brain is responsible for the increased phenotypic hierarchical classification of cortical development disorders as a cause of epilepsy in conjunction with cognitive defects and developmental delay. In addition to the imaging advances, the molecular delineation of the neuronal migration disorders have revealed correlations with steps in cortical development at stages which include migration of neurones and proliferation of neural progenitors. Epilepsy is particularly prevalent in classical lissencephaly where normal gyration of the cortex is absent or reduced and there are four cortical layers instead of the normal six layers. There are two major genes associated with lissencephaly, namely *LIS1* (platelet activating factor acetohydrolase beta subunit) and X-linked *DCX* (double-cortin) and more recently the tubulin genes (*TUBA1A, TUBA2B*). These genes work together in the biochemical pathway which regulates microtubule formation, stimulates microtubule polymerisation and neuronal motility.[17]

(c) Neuropsychiatric disorders: In the community, epilepsy is associated with an increased prevalence of mental health disorders compared with the general population. Patients with epilepsy are about two and a half times more likely to have schizophrenia and almost three times more likely to have schizophrenia-like psychosis than people without epilepsy. Epilepsy is also associated with a higher prevalence of suicidal ideation and anxiety disorders. All of these neuropsychiatric traits have very high rates of heritability (or genetic loading) and are presently being intensely investigated through the new genetic mapping techniques. The epilepsy gene-hunting laboratories would be well advised to mimic best-practice guidelines and the exhaustive efforts of the neuropsychiatric genetics work and not attempt to re-invent the wheel as the new genetics era emerges (International Schizophrenia Consortium).

5. The morning after the night before – waking up to complex genetics

Let us be honest – the epilepsy community has been spoilt by the gene-discovery in Mendelian families which unintentionally delayed the hard truth – like other complex disorders, the heritability of epilepsy on a population basis is logistically complex and driven by polygenic susceptibility loci. Application of linkage analysis to complex epilepsy has suggested various susceptibility loci, but these findings have a very poor record of replication. This approach has failed to uncover a 'common epilepsy gene', validating the concern that complex epilepsy syndromes may be too genetically heterogeneous for the proposed SNP-based disease association approach to succeed. Non-replicable case–control candidate gene (genome-wide or candidate gene/hypothesis-driven) or transmission disequilibrium tracking with disease association (SNP haplotypes) has failed to identify new genes for complex epilepsy. Association studies have traditionally been underpowered to detect any small effects of polygenes. Genetic associations with epilepsy have a poor record of replication, either because of underlying ascertainment bias, prior assumptions of the degree of heterogeneity or sensitivity to the statistical pitfalls of multiple testing.

However, it is now apparent that genome-wide searching for genes of effect is becoming more successful in the background of the genome project, the SNP HapMap resource, faster technology platforms and high-speed supercomputing. In 2007, Cavalleri et al.[18] described the first serious attempt to deliver a multi-institutional, large-population study in epilepsy genetics. Despite the lack of positive results this reflected on the issues which would make future experiments worthwhile, quoting several factors such as better standard of clinical phenotyping (and breaking down to endo-phenotypes), collection of large standardised multi-centre cohorts and access to genome centres and computing power. Furthermore, large cohorts of IGE samples are being screened through new genomic platforms such as Array-CGH panels where the search for large genome-wide deletions, insertions and duplications are underway. This has already been a successful platform in syndromic epilepsy and in case studies with intellectual disability, as well as many other disorders such as autism/ADHD, schizophrenia, and cancer.[19]

6. Epilepsy genetics – the future

In the short term the next few years will see the publication of studies which reflect the multicentre efforts in candidate gene screening, SNP array genome-wide association studies, Array CGH, as well as laboratory efforts to understand the biology of known and emerging genes. Ticking away in the background will be more gene-discovery in Mendelian families, recessive mapping of consanguineous alleles, a plethora of syndromic epilepsy co-morbid with other phenotypes, and animal models to aid our neurobiological understanding of epileptogenesis. However, times are changing and approaching rapidly on the horizon is a revolution in genetics which will initiate a new approach and

create novel outcomes in epilepsy genetics. As ever, good genetics begins with excellent phenotyping and efforts are underway to improve seizure diagnosis and define variation with phenotypes and clinical presentation. For example the deep phenotyping of CAE or JME may yield endo-phenotypic categories which will select for great genetic homogeneity and easier interpretation of downstream genetic analysis. This concept is widely adopted in other complex disorders and the 'Emperor's new clothes' will become apparent in due course.

What is changing rapidly is the application of whole-genome sequencing to medical genetics research. Although personalised WGS is still prohibitively expensive, it is the affordability of exome sequencing which is exciting, promising and daunting. Exciting because exome sequencing will sequence the entire coding regions of the majority of genes in a given genome; promising because it has the capacity to pull out the disorder-specific genotypes and reveal genetic heterogeneity/proteome implication; and daunting because the bioinformatic challenge is substantial even though it is only 1–2% of the genome and it has the capacity to define other disorders outside the focus of the study. Given the power of this approach and the requirement for small numbers of cases, it is likely that exome sequencing will have an increasing profile in epilepsy research in the next 5 years. The first discoveries using this platform are now published[20,21] and most experts agree that it is step-change in medical genetics.

Where will be sixty years from now? If we consider that 60 years ago that DNA was a fledgling science and medical genetics an embryonic clinical priority, then we can expect several evolutions and a landscape which may be too difficult to predict. The pace of change in methodologies, technology capability, bioinformatics and super-computing is already evident and so the first safe prediction is that personalised genomes will be as accessible as other pathology tests and will also compliment pathology indices by explaining the biomarker results. The second is that the Brave New World in genetics will have significant challenges during development because of the ethical dilemmas of multiple susceptibilities and health service loading on counselling services. The third is that most, if not all, of the epilepsy predisposition genes will have been identified and characterised on a cellular and physiological basis and be a standard interpretation at the clinical level. The fourth, and most important, is that interventions will be available, not necessarily drugs, rather more advances in autologous cell therapy, neural network manipulation and nano-technology. If we are still disappointingly reliant on pharmacology in 2070, then certainly the drugs will be more specific and personalised, with responsiveness and side-effect profiles revealed before administration. Lastly, it is to be hoped that health service delivery to epilepsy patients will be determined by care plans that are holistic, integrated, multi-disciplinary and not mired in politics or financial austerity, or limited by a lack of knowledge.

Conflict of interest statement

None declared.

References

1. Berkovic SF, Mulley JC, Scheffer IE, Petrou S. Human epilepsies: interaction of genetic and acquired factors. *Trends Neurosci* 2006;**29**:391–7.
2. Gourfinkel-An I, Baulac S, Nabbout R, Ruberg M, Baulac M, Brice A, et al. Monogenic idiopathic epilepsies. *Lancet Neurol* 2004;**3**:209–18.
3. Szoeke CE, Newton M, Wood JM, Goldstein D, Berkovic SF, et al. Update on pharmacogenetics in epilepsy: a brief review. *Lancet Neurol* 2006;**5**:189–96.
4. Turnbull J, Lohi H, Kearney JA, Rouleau GA, Delgado-Escueta AV, Meisler MH, et al. Sacred disease secrets revealed: the genetics of human epilepsy. *Hum Mol Genet* 2005;**14**(Spec No. 2):2491–500.
5. Kurian MA, Meyer E, Vassallo G, Morgan NV, Prakash N, Pasha S, et al. C beta 1 deficiency is associated with early-onset epileptic encephalopathy. *Brain* 2010;**133**:2964–70.
6. Kurian MA, Zhen J, Cheng SY, Li Y, Mordekar SR, Jardine P, et al. Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia. *J Clin Invest* 2009;**119**:1595–603.
7. Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, et al. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet* 2001;**28**:49–52.
8. Cope DW, Di Giovanni G, Fyson SJ, Orban G, Errington AC, Lorincz ML, et al. Enhanced tonic GABAA inhibition in typical absence epilepsy. *Nat Med* 2009;**15**:1392–8.
9. Jiruska P, Csicsvari J, Powell AD, Fox JE, Chang WC, Vreugdenhil M, et al. High-frequency network activity, global increase in neuronal activity, and synchrony expansion precede epileptic seizures in vitro. *J Neurosci* 2010;**30**:5690–701.
10. Crompton DE, Berkovic SF. The borderland of epilepsy: clinical and molecular features of phenomena that mimic epileptic seizures. *Lancet Neurol* 2009;**8**:370–81.
11. Vadlamudi L, Dibbens LM, Lawrence KM, Iona X, McMahon JM, Murrell W, et al. Timing of de novo mutagenesis—a twin study of sodium-channel mutations. *N Engl J Med* 2010;**363**:1335–40.
12. Schulte U, Thumfart JO, Klocker N, Sailer CA, Bildl W, Biniossek M, et al. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvbeta1. *Neuron* 2006;**49**:697–706.
13. Zhou YD, Lee S, Jin Z, Wright M, Smith SE, Anderson MP. Arrested maturation of excitatory synapses in autosomal dominant lateral temporal lobe epilepsy. *Nat Med* 2009;**15**:1208–14.
14. Rees MI, Harvey K, Pearce BR, Chung SK, Duguid IC, Thomas P, et al. Mutations in the gene encoding GlyT2 (SLC6A5) define a presynaptic component of human startle disease. *Nat Genet* 2006;**38**:801–6.
15. Raymond FL, Tarpey P. The genetics of mental retardation. *Hum Mol Genet* 2006;**15**(Spec No 2):R110–116.
16. Shoubridge C, Tarpey PS, Abidi F, Ramsden SL, Rujirabanjerd S, Murphy JA, et al. Mutations in the guanine nucleotide exchange factor gene IQSEC2 cause nonsyndromic intellectual disability. *Nat Genet* 2010;**42**:486–8.
17. Barkovich AJ, Millen KJ, Dobyns WB. A developmental and genetic classification for midbrain–hindbrain malformations. *Brain* 2009;**132**:3199–230.
18. Cavalleri GL, Weale ME, Shianna KV, Singh R, Lynch JM, Grinton B, et al. Multicentre search for genetic susceptibility loci in sporadic epilepsy syndrome and seizure types: a case–control study. *Lancet Neurol* 2007;**6**:970–80.
19. Williams NM, Zaharieva I, Martin A, Langley K, Mantripragada K, Fossdal R, et al. Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: a genome-wide analysis. *Lancet* 2010.
20. Bilguvar K, Ozturk AK, Louvi A, Kwan KY, Choi M, Tatli B, et al. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. *Nature* 2010;**467**:207–10.
21. Jones S, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 2009;**324**:217.